Association Between Cytomegalovirus Viremia and Incident Retinitis, Progression of Existing Lesions, and Reactivation in HIV+ Patients in the U.S.

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1. ABSTRACT

Cytomegalovirus retinitis (CMVR) is the most common ocular opportunistic infection associated with HIV/AIDS, and is becoming a major cause of blindness in regions of the world most affected by it. In addition to the startling mortality risk a diagnosis of CMVR carries, it has also been reported that CMV viremia is associated with an increased mortality risk. We examine data from the Longitudinal Study of Ocular Complications of AIDS (LSOCA) database to determine if CMV viremia as detected via PCR could be used to predict CMVR incidence, additional lesions, border advancement, and reactivation. Using mixed effects logistic regression methods, we find that CMV viremia in the blood is strongly associated with incident disease and, to a lesser but still significant extent, border advancement in the year following detection. While not statistically significant, association between CMV viremia and both the manifestation of additional lesions and CMVR lesion reactivation are suggestive and deserve further modeling considerations and additional studies in a variety of populations around the globe. Regardless, not only will this study serve as a critical marker of CMV incidence, progression, and mortality risk, but as a useful tool to triage individuals at highest risk of ocular complications in resource limited settings.

2. INTRODUCTION

2.1 Background

As of 2018, approximately 36.9 million people are living with HIV worldwide, southeast Asia and Africa the most severely affected regions [1]. Local and international efforts in these regions have successfully focused on programs for HIV diagnosis and treatment; however, most of these programs lack screening for cytomegalovirus. Cytomegalovirus (CMV), the largest member of the herpes virus family, infects people of all ages, including newborns; one out of every 200 babies are born with a congenital CMV infection and over half of adults have been infected by age 40 [2]. Using Immunoglobulin G (IgG) and Immunoglobulin M (IgM) values from blood tests, past exposure and latent infection can be detected with high accuracy [3]. Most people infected with CMV present no signs or symptoms, but it is best known to infect or reactivate in severely immunocompromised humans such as organ transplant recipients and those living with HIV/AIDS.

Cytomegalovirus retinitis (CMVR) is the most common ocular opportunistic infection associated with AIDS, and is becoming a major cause of blindness in regions of the world where HIV/AIDS is most prevalent [4]. Manifestation of CMVR begins with floaters in the eye, causing blind spots and blurred vision, eventually leading to the development of retinal lesions [5]. CMVR usually affects the peripheral retina (zones 2 and 3), but can affect the back of the eye (zone 1) as well, which is aptly named macular CMVR. CMVR progresses

insidiously and, if left untreated, will become bilateral in 80% of cases and eventually results in irreversible blindness. There is no unified approach to screening, and as a result CMVR is becoming an important cause of blindness in resource-limited regions of the world [6-8]. In the Johns Hopkins CMV Retinitis cohort, consisting of patients with AIDS and CMVR, it was shown that diagnosis of CMVR carries a startlingly high mortality risk but highly active anti-retroviral therapy (HAART) substantially reduces this risk, and systemic anti-CMV therapy reduces this risk even further - the combined effect of HAART and anti-CMV therapy reduces mortality risk by about 65% for whom HAART does not induce immunological recovery [9].

It has also been reported that CMV viremia is associated with an increased mortality risk, especially in regions where resources are scarce [10-14]. CMV viremia is a more precise quantifier of the virus' activity because it indicates active rather than latent CMV infection; virological markers of ongoing CMV replication are likely to be more accurate predictors of retinitis activity [15]. In CMV seropositive patients with AIDS, detection of CMV viremia has been shown to be predictive of death and provides prognostic information in addition to CD4 counts and HIV viral load. Prior to the era of HAART there was a significant amount of research into the utility of CMV viremia to predict CMVR onset and relapse as well as extra-ocular disease with mixed results [16-19]. Yet, a recent study has demonstrated CMV DNA PCR may have high diagnostic value for CMVR and extra-ocular disease [20]. We are interested in examining data from the Longitudinal Study of Ocular Complications of AIDS database to determine if CMV viremia as detected via PCR could be used to predict new CMVR incidence, progression of existing disease, and border advancement.

Thus, in addition to serving as a marker of mortality risk, our research may be used as part of a potential tool to triage individuals at highest risk of ocular complications in resource limited settings. Traditionally an ophthalmologist performing indirect ophthalmoscopy can diagnose CMVR in a sensitive and specific manner, but many of the countries with the largest burden of HIV have the lowest number of ophthalmologists and cannot meet the growing demand for screening evaluations. Those in need also tend to live in rural areas far from population centers where most ophthalmologists are located [21]. For example, a study found CMVR in an astounding 33% of newly diagnosed HIV patients in Northern Thailand, a region that is predominantly rural and resource-poor [22]. While novel approaches such as telemedicine and the diagnosis of CMVR by primary care providers are undergoing thorough investigation, there remains a space for further innovation to refer the most at-risk individuals to the care they require [23-25]. While the database we analyze is from the United States, we assert that the identification of CMV viremia in the blood could be a sign of CMVR complications in the near future, which, if care is provided efficiently in resource-poor communities, can reduce incidence and unfavorable evolution of the disease, and ultimately mortality.

2.2 LSOCA data

The Longitudinal Study of Ocular Complications of AIDS (LSOCA) is a prospective observational study of AIDS patients sponsored by Johns Hopkins Bloomberg School of Public Health in collaboration with the National Eye Institute (NEI) [26]. 2392 U.S.-based patients aged 13 years or older with a prior diagnosis of AIDS according to the 1993 Centers for Disease Control and Prevention (CDC) criteria with or without ocular complications were enrolled over a four year period. Follow-up visits for patients without ocular complications occur every six months, whereas if a patient has ocular complications at baseline or is diagnosed during a follow-up, visits occur every three months. Follow-up data includes eye examinations, fundus photographs, hematology and serum chemistry, visual function testing, and medical history. In addition, plasma and blood cell specimens were collected for banking, the analysis of which includes HIV RNA levels and CMV DNA levels. The purpose of this data is to monitor longitudinal trends in the incidence of CMVR and other ocular complications of AIDS, to determine the effect of HAART-induced immune status on ocular disease risk, to determine the clinical, virologic, and hematologic characteristics of a population at high risk for ocular complications of AIDS, and to evaluate the effects of treatments for ocular complications on visual function, quality of life, and survival.

A subset of the full data was taken; clinical data was selected for patients with at least one study visit as of 8/1/2004 whether they had PCR testing for CMV viremia or not. Two datasets adhering to this condition were available to use in the analysis, both without any demographic data of the patients such as age, gender, or race. The first is the data collected from clinics, visit-specific data including CD4, CD8, and HIV viral load counts, PCR tests for CMV viremia, and indicator variables for IV and local CMV therapies, including valganciclovir, ganciclovir, cidofovir, and foscarnet. The second is a collection of fundus photograph data from reading centers - eye-specific activity, movement, and new lesion variables, as well as the binary variable indicating if an eye has CMVR. This binary variable was generated by the analysis team at the coordinating center, drawing upon multiple sources of information including clinician assessments; activity and other variables in the dataset are scored by the reading center based only on fundus photographs. The activity score and the CMVR indicator variable do not necessarily agree because fundus photographs do not include the most anterior portion of zone 3, which can be seen by clinicians and would fail to be identified by the reading center.

2.3 Hypothesis

We hypothesize that positive PCR tests for CMV viremia predict incident disease or additional lesions, one of the criteria for progression, in the 12 months after detection. We also hypothesize that presence of CMV viremia is, at best, mildly associated with border advancement, the other criteria for progression, and reactivation of pre-existing lesions in the 12 months after detection.

3. METHODS

3.1 Data summary

The subset of data has 1763 patients, nine of whom have no available reading center data. Each patient has a mean of six visits including baseline (median 5, IQR 3-8, minimum 1, maximum 22). 1269 (72.0%) patients have more than one visit at which eye information is unknown - in other words, for a majority of patients, it is uncertain to which eye the data belongs for one or more visits. Fortunately, 1752 (99.4%) patients have complete eye information at baseline.

366 (20.8%) patients have CMVR at some point during the course of the study, whereas 1388 (78.7%) do not, and 9 (0.5%) have no such information from the reading center (Table 1). 115 (6.5%) patients have at least one positive PCR test for CMV viremia, 1479 (83.9%) have all negative PCR tests, and 169 (9.6%) patients had no PCR tests at all during the course of the study period. Given that a patient had CMVR at one or more visits, 71 (19.4%) had at least one positive PCR test for CMV viremia (visits with CMVR and CMV viremia did not necessarily coincide). If a patient never had CMVR over the course of the study, only 44 (3.2%) had at least one positive PCR test for CMV viremia. For those 115 patients who had any positive tests for CMV viremia, just 30 (26.1%) had more than one over the study period, with a maximum of 5 positive PCR tests for one patient.

The 115 patients who test positive for CMV viremia at least once have lower mean CD4 (95.2 vs 278.1) and CD8 counts (502.4 vs 877.2) on average and higher mean HIV viral load counts (4.5 vs 3.2) on average compared to those who never tested positive for CMV viremia. These values are means of within-patient means over the course of the study. The visits at which there was a positive PCR test had lower CD4 (mean 55.8, median 20) and CD8 counts (mean 418.3, median 282), and higher HIV viral load counts (mean 4.8, median 5.2) compared to the visits at which there was no positive PCR test for CMV viremia (CD4: mean 288.0, median 246; CD8: mean 884.7, median 788; VL: mean 3.1, median 2.6).

Out of the 1688 patients who have available new lesion data, 32 (1.9%) patients developed new CMVR lesions during the study period, incident disease or otherwise. All 32 of these patients had lesions develop in the mid- or far periphery (retinal zones 2 and 3) while only 11 (34.4%) had macular CMVR (retinal zone 1) develop (Table 2). Out of the 1751 patients that have activity data available, 239 (13.6%) had inactivity but no active CMVR, and 141 (8.1%) had mild or severe CMVR lesions. Note that in every instance, new lesions and activity happen more often in the peripheral retina compared to the macula, which is expected based on the literature. 1688 patients have available border advancement data; of these, 78 (4.6%) have at or over 750 microns of movement over the study period. Border advancement appears to be less prevalent in macular CMVR; of the 36 patients who had border advancement in retinal zone 1, only 12 (33.3%) had a lesion with at least 3000 microns of

advancement, whereas out of the 72 who had border advancement in the peripheral retina, 39 (54.2%) had a lesion with at least 3000 microns of advancement. Unconditional on retinal zone, though, it was more common to have at least 3000 microns of border advancement over the course of the study (52.6%) than to have less (47.4%) given a patient had any at all.

It should be noted that 281 (15.9%) patients underwent IV therapy for CMV at one or more visits, while 31 (1.8%) underwent local therapy for CMV in the form of injection or implant at one or more visits (data not shown). While this data did not play an active role in the forthcoming analysis, it is an important consideration when discussing limitations later.

3.2 Development of risk sets

In order to determine unbiased estimates of the effect of CMV viremia on CMVR-related activity, the appropriate patients and visits must be identified, isolated, and analyzed separately from the rest of the data. Pinpointing these valid risk sets, defined as subsets of patients and visits therein that are at risk for a specific well-characterized event, is of paramount importance to generating correct odds ratios.

We are only interested in the presence of CMV viremia in the year preceding any given visit, so once a risk set for each analysis is determined, an R function iterates through each patient and visit, iteratively building a new dataset. Each dataset includes the outcome variable for the particular analysis, a binary covariate indicating whether or not CMV viremia was detected at that visit or in the previous 365 days, and CD4 count, a strong barometer of immunosuppression, as a confounding factor. Also included in each newly created dataset are any categorical variables that indicate if a visit meets some inclusion or exclusion criteria; after the dataset is created in full, visits to be excluded can be removed.

3.2.1 Incident CMVR risk set

First, we need to determine the set of patients who are at risk of developing CMVR. A patient's IgG measure is taken only at baseline, so we assert that a patient is CMV+ if their baseline IgG measure is at least 1.2 IU/mL or if they present with CMVR at some point in the study. Those who are confirmed to be CMV- at baseline and develop CMVR later should also be included; either the IgG test was a false negative or the patient attained CMV at a visit post-baseline and before their first CMVR lesion manifested. There are 50 such patients who were tested to be IgG- at baseline, 3 of whom presented with CMVR at later follow-ups.

The union of the 1173 patients confirmed to be IgG+ at baseline and the 366 CMVR+ patients makes for an initial count of 1269 patients at risk for CMVR. As we are interested in incident disease, we must take the intersection of our current group of 1269 and the 1407 patients who have no activity at baseline, defined as those

who have no active or inactive lesions upon enrollment, totaling 924 patients. After visits are removed with an unknown new lesion measure or that occur after incident disease occurs, 868 patients remain. Lastly, visits are removed that have zero tests for viremia in the previous year to remove additional bias; 768 patients remain, and are those who are included in the final incident CMVR risk set. It should be noted that because we are looking for incident disease - that is, the first visit at which CMVR presents in either eye in a given patient - we do not separate eyes in this analysis.

3.2.2 Additional lesions risk set

To be at risk for additional CMVR lesions, a patient must have active CMVR at baseline or active lesions before enrollment, indicated by inactive lesions at baseline. There are 340 patients who fit this description. However, we need to consider the 12 patients in the study who had incident CMVR, 10 of whom had visits after the incident disease occurred (the other two had incident CMVR in their last known follow-up). Thus, visits from 350 patients are considered in this analysis. After visits are removed with an unknown new lesion measure or that have zero tests for CMV viremia in the past year, 323 patients remain. When analyzing eyes separately, rows without available eye data were removed.

3.2.3 Border advancement risk set

The risk set for border advancement is all patients who currently have active or inactive lesions. It is true that if a lesion is inactive, borders cannot advance. However, these lesions may become active in between visits and borders may advance before the lesions become inactive again - in fact, 21 visits in the dataset show border advancement occurring when a lesion is labelled inactive or questionable. Visits from a total of 380 patients are considered in this analysis, but after some are removed with an unknown border advancement measure or that have zero tests for CMV viremia in the past year, 350 patients remain. When analyzing eyes separately, rows without available eye data were removed.

3.2.4 Reactivation risk set

Reactivation requires an existing inactive lesion, so the risk set is all patients who have lesions of inactive or questionable status at any point in the study such that the reactivated lesion is not reported as new, which would not constitute a pre-existing lesion. However, there are many instances where a patient with an inactive lesion reports border advancement but no activity, which implies reactivation between visits; this additional condition is considered as a new reactivation indicator variable is created. Visits from 380 patients are considered before some are removed with an unknown reactivation variable measure or that have zero tests for CMV viremia in the past year - 325 patients remain in the risk set. When analyzing eyes separately, rows without available eye data were removed.

3.3 Mixed effects logistic regression

For each risk set, we use the R function *glmer* from the *lme*4 package to perform mixed effects logistic regression, with the binary outcome regressed on the binary variable indicating if CMV viremia was present in the previous year, CD4 count, a strong indication of immunosuppression, as a confounding variable, and a random effect term.

There are four ways to model the random effect. The first is to combine data for left and right eyes per visit per patient; that is, we take the maximum state between eyes at each visit such that events take precedent over non-events. The second is to treat eyes as separate units per patient, but imply no correlation between eyes on the same patient. The third is to create ID/eye clusters such that the correlation structure only applies to visits in the same eye on the same patient, but does not group eyes of the same patient in any way. The fourth, and preferred, method is to nest left and right eyes within patient ID. We report results using all four methods to demonstrate sensitivity and concordance between estimates. When CD4 count is included in models, it is standardized to improve convergence behavior. We used bound optimization by quadratic approximation to further promote model convergence.

4. RESULTS

4.1 Incident CMVR

Within the risk set, there were a total of 12 visits at which the patient presented with incident disease (1 with unknown CMV viremia status in the year previous) and 40 visits at which the patient had been viremic in the past year based on at least one PCR test. The proportion of visits where there was a new lesion within a year of being viremic is 6 of 40 (15.0%), whereas if a patient was not viremic in the previous year, the proportion of visits where a patient presented with incident disease within a year is 5 of 1207 (0.4%) (Table 3). Having at least one positive PCR test for CMV viremia increases odds of incident CMVR in the following year by a factor of 12.14 compared to those who did not have any positive PCR tests in the previous year, adjusting for CD4 count (95% Wald CI: (3.41, 43.21), $p < 0.001$) (Table 9). Not adjusting for CD4 count inflates the odds ratio estimate to 42.42.

4.2 Additional lesions

Within our at-risk patient's visits, there were a total of 27 visits at which a patient presented with new lesions that were not incident disease and 195 visits at which the patient had been viremic in the past year based on at least one PCR test. The proportion of visits where there was a new non-incident lesion within a year of being viremic is 11 of 195 (5.6%), whereas if a patient was not viremic in the previous year, the proportion of visits

where a patient presented with a new non-incident lesion within a year is 16 of 2372 (0.7%) (Table 4). 9 eyes presented with additional lesions of the 347 (2.6%) that had viremia in the previous year, whereas 14 eyes had new lesions of the 4493 (0.3%) that did not have viremia in the previous year (Table 5). Having at least one positive PCR test for CMV viremia increases odds of additional CMVR lesions in the following year by a factor of 2.15 compared to those who did not have any positive PCR tests, adjusting for CD4 count and nesting eye within patient (95% Wald CI: (0.58, 7.95), $p = 0.250$) (Table 9). The other three model specifications, whether or not they adjusted for CD4 count, agreed with the direction and, generally, the magnitude of the association.

4.3 Border advancement

In the risk set outlined previously, there were a total of 126 visits at which a patient showed a detectable level of border advancement relative to the previous visit and 205 visits at which a patient had been viremic in the past year based on at least one PCR test. The proportion of visits where there was border advancement within a year of being viremic was 40 of 205 (19.5%), whereas if a patient was not viremic in the previous year, the proportion of visits where border advancement was evident within a year was 79 of 2419 (3.3%) (Table 6). 40 eyes presented with border advancement of the 287 (13.9%) that had viremia in the previous year, whereas 85 eyes had border advancement of the 3152 (2.7%) that did not have viremia in the previous year (Table 7). Having at least one positive PCR test for CMV viremia increases odds of border advancement in the following year by a factor of 1.96 compared to those who did not have any positive PCR tests, adjusting for CD4 count and nesting eye within patient (95% Wald CI: (0.98, 3.91), $p = 0.058$) (Table 9). The other three model specifications, whether or not they adjusted for CD4 count, agreed with the direction of association and the magnitude of the statistical significance, in general.

4.4 Reactivation

Out of all patients at risk for CMVR reactivation, there were a total of 62 eyes in which reactivation was detected and 196 eyes that had presented with CMV viremia in the past year based on at least one PCR test. The proportion of eyes in which reactivation was detected within a year of being viremic was 14 of 196 (7.1%), whereas if a patient was not viremic in the previous year, the proportion of visits where reactivation was evident within a year was 44 of 2938 (1.5%) (Table 8). Having at least one positive PCR test for CMV viremia increases odds of CMVR reactivation in the following year by a factor of 1.95 compared to those who do not have any positive PCR tests, adjusting for CD4 count and nesting eye within patient (95% Wald CI: (0.49, 7.79), $p = 0.344$) (Table 9). Model specifications that did not adjust for CD4 count report odds ratios almost three times as large as those that did, and despite wide 95% confidence intervals, statistical significance at the 0.05 level is achieved if CD4 count is not adjusted for.

5. DISCUSSION

CMV viremia is strongly associated with incident CMVR and, to a lesser extent, border advancement in the year following detection. While not statistically significant, association between CMV viremia and both the manifestation of additional lesions and reactivation of lesions are weakly suggestive. This study provides evidence that CMV viremia is a strong indicator for retinitis incidence in HIV+ patients infected with CMV, without implementing additional analytical strategies as discussed below. Given these encouraging results, we think that with access to more data sources and collaboration with international communities in resource limited settings, the development of programs to screen HIV-infected individuals for CMV and CMV viremia to identify those in a high risk pool will curtail a significant fraction of ocular complications as a result of HIV/AIDS and retinitis.

One major caveat of the analysis as it stands now is that any significance in current or future border advancement and reactivation analyses could be related to the CMV already in the pre-existing lesion, so it may not depend on new virus traveling to the eye through the bloodstream. The association seen in the border advancement analysis is most likely an indirect relationship, with both the CMV viremia and the enlargement of lesions reflecting worsening immune function that allows reactivation of the virus in the retinal lesions and for reactivation of virus in other organs, resulting in viremia. Another, although less likely, explanation for this relationship is that tissue damage in pre-existing lesions allows circulating virus to spread to previously uninfected retinal tissue at the lesion borders, making it appear that the border is reactivating from virus already there, when in fact the perceived reactivation is actually a new lesion at the border attributable to newly arrived virus. An argument against the latter possibility is the fact that reactivation generally occurs along a long segment of the border, whereas a new lesion on the border would likely be an isolated focus. We do not have information from the LSOCA dataset to address any of these possibilities, so additional, carefully considered research would need to be done with data from an alternative source.

Several future amendments to the models presented in this study to strengthen conclusions, both small- and large-scale, are warranted. First, it is wise to adjust for HIV viral load and use of CMV therapies in addition to CD4 count. Second, not only should we use the continuous values of the CMV viremia concentration in the blood if it is present, but we should weight positive PCR tests according to their recency relative to the visit of interest, making the variable a time-dependent covariate. Third, using missing data methods such as multiple imputation would increase the number of patients in the aforementioned risk sets and make estimates more precise. Lastly, we should try to use the slew of similar papers published in the past two decades regarding CMV viremia to inform prior distributions, setting the stage for a set of full-fledged Bayesian models.

The completed analysis shown here is preliminary and needs a few notable improvements to strengthen

its story. However, what has been revealed is promising; CMV viremia appears to be a reliable indicator of future CMVR incidence and some CMVR activity, a fact that can be used to provide informed, structured, and individualized care in the U.S. and abroad, reducing mortality and improving quality of life for those afflicted with HIV/AIDS.

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7. APPENDIX

+ viremia tests	CMVR+	CMVR-	N/A	Total
0	270	1202	7	1479
>0	71	44	0	115
1	*46*	*39*	*0*	*85*
2	*18*	*4*	*0*	*22*
3	*5*	*1*	*0*	*6*
4	*1*	*0*	*0*	*1*
5	*1*	*0*	*0*	*1*
N/A	25	142	2	169
Total	366	1388	9	1763

Table 1. Patients with and without CMVR stratified by number of positive PCR tests for viremia

	Zone 1	Zone 2/3	All zones
New lesion relative to previous visit			
Yes	11	32	32
No	1677	1656	1656
N/A	75	75	75
Most severe CMVR activity achieved			
None	1558	1365	1371
Inactive	123	241	239
Mild	29	54	56
Severe	41	85	85
N/A	12	18	12
Max border advancement, microns			
No movement	1652	1616	1610
< 750	10	16	17
[750, 1500)	10	6	9
[1500, 3000)	4	11	11
≥ 3000	12	39	41
N/A	75	75	75

Table 2. Frequency of patients with CMVR-related events in study period stratified by retinal zone

	Viremia in the previous year		
Incident CMVR	No	Yes	Unknown
No	1202	34	172
Yes	5	6	1

Table 3. Visits at risk for incident CMVR stratified by viremia status in the year previous

	Viremia in the previous year		
Additional lesions	No	Yes	Unknown
No	2356	184	339
Yes	16	11	0

Table 4. Visits at risk for additional CMVR lesions stratified by viremia status in the year previous

	Viremia in the previous year		
Additional lesions	No	Yes	Unknown
No	4479	338	674
Yes	14	9	1

Table 5. Eyes at risk for additional CMVR lesions stratified by viremia status in the year previous

	Viremia in the previous year		
Border advancement	No	Yes	Unknown
No	2340	165	331
Yes	79	40	7

Table 6. Visits at risk for border advancement of CMVR lesions stratified by viremia status in the year previous

	Viremia in the previous year		
Border advancement	No	Yes	Unknown
No	3067	247	459
Yes	85	40	14

Table 7. Eyes at risk for border advancement of CMVR lesions stratified by viremia status in the year previous

	Viremia in the previous year		
Reactivation	No	Yes	Unknown
No	2894	182	229
Yes	44	14	4

Table 8. Eyes at risk for reactivation of CMVR lesions stratified by viremia status in the year previous

Incident CMVR					
		CD4 adjustment		No CD4 adjustment	
Data	**Random effect**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Combine eyes per patient	Patient only	12.140 (3.411, 43.207)	<0.001	42.423 (12.341, 145.839)	<0.001
Additional lesions					
Combine eyes per patient	Patient only	1.979 (0.856, 4.754)	0.110	1.318 (0.317, 5.477)	0.704
Treat eyes separately	Patient only	2.209 (0.692, 7.055)	0.181	2.332 (0.546, 9.960)	0.253
	Patient/eye clusters	2.363 (0.976, 5.723)	0.057	2.606 (0.573, 11.855)	0.215
	Nest eye in patient	2.153 (0.583, 7.951)	0.250	2.416 (0.544, 10.737)	0.246
Border advancement					
Combine eyes per patient	Patient only	3.069 (1.582, 5.954)	<0.001	4.240 (1.776, 10.125)	0.001
Treat eyes separately	Patient only	1.877 (0.957, 3.682)	0.067	2.013 (0.905, 4.476)	0.086
	Patient/eye clusters	2.283 (1.180, 4.416)	0.014	2.731 (1.191, 6.262)	0.018
	Nest eye in patient	1.955 (0.977, 3.912)	0.058	2.300 (0.996, 5.308)	0.051
Reactivation					
Treat eyes separately	Patient only	1.910 (0.538, 6.783)	0.317	4.145 (1.148, 14.975)	0.030
	Patient/eye clusters	2.066 (0.528, 8.075)	0.297	5.404 (1.446, 20.190)	0.012
	Nest eye in patient	1.951 (0.489, 7.785)	0.344	5.305*	

* - Model did not converge; only point estimate provided

Table 9. Results of mixed effects logistic regression models

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